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K. S. Rajan

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The objective of this research is to investigate feasibility of immobilizing squid-type DFPase on fabrics such as nylon, polyester, cotton and polyester-cotton blends and on polyethylene glycol. Large quantities (about 15,000 units) of the enzyme was prepared from the hepatopancreas tissues of East Coast (ECHP) and West Coast (WCHP) squid belonging to the species, Loligo Pealii and Loligo Opalescens. The purified enzyme preparations were characterized by their activities against diisopropylphosphorofluoridate (DFP), isopropylmethyl phosphonofluoridate (SARIN) and pinacolylmethyl phosphonofluoridate (SOMAN) and their kinetic constants ($K_M^{\wedge}$ and $V_{MAX}^{\wedge}$). The enzyme-immobilization study included

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ABSTRACT (Continued)

the exploration of different preactivation methods involving treatment with (i) glutaraldehyde, (ii) triethyloxoniumtetrafluorobcrate (TTFB), (iii) trasyl-chloride, (iv) isocyanide, (v) cyanuric chloride and (vi) $KMnO_4$-oxidation. Results of examination of the DFPase-immobilized matrices showed agent-specific activities (DFP, SOMAN, and SARIN) in the range 3.5 to 17.7 units/gram. A number of them retained up to 90% of their initial enzyme activity over a 90-day period. This is indicative of a good potential for this technical approach squid-type DFPase.

Progress Report on Contract No. N00014-89-K-0054
IITRI Project No. C06692

Principal Investigator: Dr. K. S. Rajan
Contractor: IIT Research Institute
Chicago, IL 60616

Contract Title: ENZYMATIC DETOXIFICATION OF CHEMICAL WARFARE AGENTS: IMMOBILIZATION OF THE ENZYME ON MATERIAL SURFACES

Research Objective:

The overall objective of this research is to investigate the feasibility of immobilizing the agent-specific enzyme, DFPase, on fabrics and one or more suitable matrices that can be subsequently used in an aerosolized form for detoxifying toxic agents such as diisopropylphosphorofluoridate (DFP), isoproplymethylphonofluoridate (SOMAN) and pinacolylmethylphosphonofluoridate (SOMAN). The specific objectives of this research are:

(i) to prepare sufficient quantities of the enzyme, DFPase, from the hepatopancreas (HP) tissues of squid belonging to the species, Loligo Pealii (East Coast Squid, ECHP) and Loligo Opalescens (West Coast Squid, WCHP);

(ii) to carry out enzyme immobilization experiments on polyester, nylon, and cotton fabrics and polyethylene glycol;

(iii) to determine the nature and extent of the enzyme immobilization achieved and to establish optimal procedures;

(iv) to determine a preliminary agent-detoxification profile of each of the enzyme-immobilized matrix samples prepared in (2) above;

(v) to initiate a study on the stability of the immobilized enzyme.

PROGRESS

ENZYME PREPARATION

The enzyme, squid-type DFPase, was prepared in substantial quantities from the hepatopancreas tissues of WCHP and ECHP by means of the procedure established earlier by this group. Their DFP-, SOMAN-, and SARIN-detoxifying characteristics

were determined by using the fluoride electrode method which involves the determination of the concentration of the F^- ion released by the reaction of DFPase on the substrate, i.e., DFP, SARIN and SOMAN. Briefly, the method of preparing DFPase consisted of the following. The tissue homogenates of the ECHP and WCHP were subjected to centrifugation to remove debris followed by ammonium sulfate precipitation, P-30 gel filtration and DEAE column chromatographic separations. The "agent-active" fractions from the P-30 gel filtration column were concentrated by ultra filtration, applied to DEAE Sepharose column and eluted with a 0 to 0.3 M NaCl gradient in bistrispropane (BTP) buffer. Eluate fractions containing the purified DFPases were collected and analyzed for their enzyme activity and protein contents. The kinetic constants, K_M and V_{MAX} for the DFPase preparations were 3.3×10^{-2}M and 39 μ moles/min/mg protein respectively for SOMAN and 4.3×10^{-3}M and 110 μ moles/min/mg protein for DFP. The average molecular weight of the DFPase preparation determined by polyacrylamide gel electrophoresis was in the range 30-36,000 Daltons.

IMMOBILIZATION OF DFPase

Enzyme immobilization studies were carried out on the following materials: nylon, polyester, cotton, and polyester/cotton blend fabrics and polyethylene glycol. Samples of each of the above materials were pretreated and "activated" before "anchoring" on the enzyme. A number of different methods were employed for chemically preactivating the matrix materials, chief among which were the following: (i) glutaraldehyde, (ii) triethyloxonium tetrafluoroborate (TTFB), (iii) tresyl chloride, (iv) isocyanide derivative, (v) cyanuric chloride and (vi) $KMnO_4$-oxidation. Selected conditions for the pre-activation of the matrices such as (i) the concentration of the preactivating chemical, (ii) duration of the preactivation reaction and (iii) temperature and their effects on the extent of immobilization of DFPase were investigated.

Results of this exploratory study appeared very promising and they indicated a need for a systematic investigation of this approach. Average values of the agent-specific activities of the different enzyme-immobilized matrices are briefly summarized below:

(i) DFPase immobilized on nylon preactivated with glutaraldehyde using polyethyleneimine spacer showed an activity of 11.0 ± 2.4 units;

(ii) By TTFB-preactivation, a maximum value of 17.1 units/g of nylon filter matrix were obtained;

(III) By tresylchloryl preactivation, DFPase-immobilized cotton fabric matrices showed activities in the range 3.5 to 5.9 units/g; and

(iv) DFP-immobilized cotton duck samples showed a maximum activity of 7 units/g.

(NOTE: 1 unit = μ moles Agent hydrolyzed/min/mg enzyme protein.)

Studies were initiated on the storage stabilities of the enzyme-immobilized fabric matrices. Preliminary results indicated that the immobilized DFPase retained up to 90% of its initial activity over an 80-90 day period.

RELATED PUBLICATIONS AND REPORTS

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2. "The Purification and Characterization of DFPases for Detoxifying Organophosphate-Type AChE Inhibitors", K. S. Rajan, et al., 1987 Annual Meeting of the American Society for Pharmacology and Experimental Therapeutics, Aug. 15-19, 1987, Honolulu, Hawaii.

3. "CW-Agent Detoxification Characteristics of Squid-Type DFPases from West Coast (Loligo opalenescens) and East Coast (Loligo pealii) Squid," K. S. Rajan, et al., 1987 Scientific Conference on Chemical Defense Research, Nov. 17-20, 1987, Aberdeen Proving Ground, Maryland; CREC-SP-88013, Vol. I, pp. 125-132, April 1988.

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7. "Molecular Topography of Squid OPA-anhydrase (EC 3.1.8.1) as Revealed by Spectroscopic Studies," J. E. Walker, J. J. Connolly, R. M. Steeves, F. C. G. Hoskin and K. S. Rajan, Proceedings of the 1989 U.S. Army CRDEC Scientific Conference on Chemical Defense Research; CRDEC-SP-024, pp. 645-651, 1990.

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